

C2 Fig 3: Alignment of CRAM-1 (SEQ ID NO.:13) and CRAM-2 (SEQ ID NO.:14)

amino acid sequences. Gaps are indicated as dashed lines.

Replace the paragraph at page 8, lines 24-27, with:

C3 Fig 6: Nucleic acid sequence of human CRAM-1 (upper panel, SEQ ID NO.: 17), complete amino acid sequence of human CRAM-1 (middle panel, SEQ ID NO.:15), and partial amino acid sequence of human CRAM-2 (SEQ ID NO.: 16).

Replace the paragraph at page 8, lines 28-38, with:

C4 Fig 7: Targeted differential display using degenerated primers. (A): Nucleotide sequences of PCR primers encoding the sequences present in C2 Ig domains are shown. Two primers encode the same sequence due to the codons encoding Ser residue. The level of degeneracy is 4096 different forms for the primers encoding YRCXAS (SEQ ID NO.: 18) and 2048 forms for the others. (B): The display of radioactive PCR products obtained with the YYCXAS1 (SEQ ID NO.: 20) primers is shown. The lanes correspond to the display of PCR product run on cDNA obtained from the t-end endothelial cell line (lane t-end), the B16 melanoma cell

Replace the paragraph at page 9, lines 18-26, with:

C5 Fig 9: JAM (SEQ ID NO.: 21), CRAM-1 and CRAM-2 murine protein sequence alignment. The identical residues are shaded in gray. The overall identity is 36% between CRAM-2 and CRAM-1, 31% between JAM and CRAM-1 and 33% between JAM and CRAM-2; the respective homologies are 52%, 52% and 49%. The gaps are shown by dashes

C5 in the sequences. The canonical conserved residues (Cys and Trp) of the V and C2 domains are marked by an asterisk.

Replace the paragraph at page 14, lines 1-24, with:

342 For 64 hours in 10 cm tissue culture dishes. As control, 5×10^5 t.End.1 and 2.5×10^5 B16 F10 cells were grown separately under the same conditions resulting in confluent monolayers after 64 hours. Total RNA was directly extracted in petri dishes with Trizol reagent following manufacturer's instructions (Gibco BRL, Paisley, Scotland). The cDNA was prepared from 5 μ g of total RNA, employing oligo-dT (16-mer) primer and Superscript Reverse Transcriptase (Gibco BRL, Paisley, Scotland). The quality and the quantity of cDNA were checked by running 27 cycles of PCR on 1 μ l of cDNA diluted 1:5, using primers specific for the housekeeping HPRT cDNA. Then the differential PCR was performed with the following degenerate primers: 5'TAYAGNTGYNNNGCYTCYAA^{3'} (SEQ ID NO.: 1), 5'TAYCRGTGYNNNGCYTCYAA^{3'} (SEQ ID NO.: 2), and 5'TAYTAYTGYNNNGCYTCYAA^{3'} (SEQ ID NO.: 3), encoding for the most frequent amino acid sequences encountered in C2 domains: YRCXAS (SEQ ID NO.:18), YQCXAS (SEQ ID NO.:19), and YYCXAS (SEQ ID NO.:20). The PCR conditions consisted of using 2 μ l of diluted cDNA; 2.5 μ l of 10X Goldstar PCR buffer; 2 μ l of $MgCl_2$; 2 μ l of degenerated primers 0.3 mM; 0.5 μ l of dNTP 0.1mM; 0.1 μ l of α^{33} dATP 10 mCi/ml (Amersham Pharmacia Biotech, Dubendorf, Switzerland); 15.65 μ l H_2O ; 0.25 μ l Goldstar Taq polymerase (Eurogentech, Seraing, Belgium).

Replace the paragraph at page 15, lines 24-38 with:

The three primers used were designed based on the EST sequences as follows:

C7 5'- GAGGTACTTGCATGTGCT-3' (SEQ ID NO.: 4) for synthesis of the first strand,

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5'-CGACAGGTGTCAGATAACA³' (SEQ ID NO.: 5) and
5'-CACCTCCTCACTCGT³' (SEQ ID NO.: 6) for the two nested PCRs. The 5' RACE-PCR product was cloned into pGem-T vector. To obtain the full length coding sequence for CRAM-1, the cloned 5'RACE-PCR product and the EST (accession No. AA726206) were digested with HpaI and NotI restriction enzymes and ligated into pGem-t vector. Cloning of full length CRAM-2 was based on the same strategy of sequence comparison and 5'RACE technique. The full-length cDNA encoding CRAM-2 was finally obtained from ESTs accession numbers: AA690843 and W80145. These two clones differ by the length of the 3' untranslated region.

Replace the paragraph at page 16, lines 26-35, with:

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Semi-quantitative PCR reaction or Northern blotting were used to determine relative amount of transcript in the various conditions. For the detection of the JAM-2 transcript, the 5'-GACTCACAGACAAGTGAC-³' (SEQ ID NO.: 7) and 5'-CACCTCCTCACTCGT-³' primer pair was used, giving a 750 bp amplification product. As internal control, the following primers specific for Hprt cDNA were used to amplify a 350/bp long fragment: 5'-GTTGGATACAGGCCAGACTTTGTTG-³' (SEQ ID NO.:9) and 5'-GAGGGTAGGCTGGCCTATAGGCT-³' (SEQ ID NO.:10).

Replace the paragraph at page 23, lines 22-36, with:

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The putative structure of the murine CRAM-1 protein is shown in Fig 8B and consists of an extracellular region with a variable heavy chain and a constant type 2 like immunoglobulin domain (Pfam, The Sanger Centre and Blast) with two potential N-linked glycosylation sites (aa 104 and 192). The hydrophobicity analysis (tmPred, ISREC) predicted a transmembrane region between positions 242-260. The postulated cytoplasmic domain